freezing point depression by nine different solutes (at the concentration range of 0.02–0.05 m) yielded an average of $K_f = 6.53$. Values for all solutes, some as different from each other as Th^{4+}, Mg^{2+}, and ClO_4^- fell within the range of 6.4–6.7. Three solutions of Mo(IV) in this solvent were prepared by absorption of Mo(IV) on a Dowex 50 X2 cation-exchange column followed by elution with eutectic acid and dilution to the desired concentration. The freezing point of each solution was measured four times by the procedure described in ref 8. The cryoscopic constants calculated from these measurements were 3.4 ± 0.1, 3.4 ± 0.2, and 3.2 ± 0.2 for solutions which were 0.10, 0.06, and 0.04 m in Mo(IV), respectively (gram-atoms of molybdenum per kilogram of solvent). The average value $K_f = 3.37 \pm 0.08$ derived from these data is 0.52 ± 0.01, or very nearly one-half of the average molal freezing-point depression of a mononuclear ion.

Sykes and co-workers[1] deduce from results of a kinetic study of the equilibrium reaction between Mo(IV) and NCS^- that this system should be presented by the equation

$$\text{Mo(IV) monomer} + NCS^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (Mo^{IV})NCS$$

They support this assumption by an ion exchange separation and analysis of the reaction product which revealed Mo:NCS ratios of 1:0.95. Ion exchange separation and analysis experiments carried out in this laboratory established that the reactants Mo(IV) and NCS^- (in 1 M acid) were in equilibrium with *two* complexes, rather than one, in the range of concentrations employed by Sykes et al.[1] in their kinetic studies. When an excess of Mo(IV) was employed the main product was "complex I" with a Mo:NCS ratio of 2:1. With NCS^- in excess, the main product was "complex II" with a Mo:NCS ratio of 1:1. With both reactants in approximately equal concentrations both complexes could be isolated in good yields along with some unreacted Mo(IV) aquo ion, as in the following experiment.

A solution containing $(NCS^-) = 5 \times 10^{-3}$ M, Mo(IV) = 4×10^{-3} M, and $(H^+) = 0.3$ M was equilibrated for 30 min at 25 °C before absorption on an ice-cooled cation exchange column (Dowex 50 X2; 100–200 mesh). Elution with 0.5 M acid revealed three distinct bands: a diffuse brown band which was eluted by this acid (complex II), a sharp brown band which was eluted with 1 M acid (complex I), and a sharp red band of the free aquo ion[2] which was eluted with 3 M acid.

Complex I conforms to a composition $(MoO)_{2n}(NCS)_n^{3n+}$. This composition is incompatible with a mononuclear structure. The only reasonable choice of n is $n = 1$, i.e., $(MoO)_2NCS^{3+}$ (or $Mo_2O_2NCS^{3+}$). The charge 3+ of this ion accounts well for its slow elution by 1 M acid. Complex II conforms to a composition of $(MoO)_m(NCS)_m^{m+}$. One could hardly justify the choice $m = 1$ since the corresponding monopositive ion $MoONCS^+$ would not be absorbed as a distinct band on an ion exchange column from a strongly acidic solution which contains a 100-fold excess of (H^+). A binuclear structure ($m = 2$), i.e., $Mo_2O_2(NCS)_2^{2+}$, conforms to the observed interaction with the ion-exchange resin as well as to the structure of complex I. We conclude that the system Mo(IV) + NCS^- under the conditions of the kinetic study[1] involves the following stepwise equilibria:

$$Mo_2O_2^{4+} + NCS^- \rightleftarrows Mo_2O_2NCS^{3+}$$

$$Mo_2O_2NCS^{3+} + NCS^- \rightleftarrows Mo_2O_2(NCS)_2^{2+}$$

This scheme does not conform to the kinetic results of Sykes et al.[1] which were obtained by employing the solutions of the reagents in perchloric acid.

Previous work in this laboratory was restricted to solutions of Mo(IV) in p-toluenesulfonic acid (HPTS),[2] except for one determination (of the oxidation state) which was conducted at 0 °C in perchloric acid. The use of $HClO_4$ was discontinued after preliminary tests demonstrated that Mo(IV) is oxidized by it to an appreciable extent at ambient temperatures (even in acid concentrations as low as 0.3 M). The lack of relevant experimental details in the paper by Sykes and co-workers[1] (such as the method of preparation and duration of storage of Mo(IV) solutions) makes it difficult to estimate the effect of $HClO_4$ on the kinetic results and the charge per molybdenum ion determination. However, their detailed account of the determination of the charge per Mo atom enables us to offer an explanation for the discrepancy between their reported experimental result, +2.20 per atom, and the expected value, +2.00. In the original determination of the charge per Mo(IV) atom by an ion-exchange saturation procedure, Ardon and Pernick[2] reported a charge per atom of 2.0 ± 0.1. Sykes and co-workers[1] repeated this procedure with $HClO_4$ and obtained a value of 2.20 which they regarded as an upper limit to the true value (2.00). A partial oxidation of Mo(IV) by $HClO_4$ in this experiment would decrease its concentration and consequently increase the apparent oxidation number.

References and Notes

(1) T. Ramasami, R. S. Taylor, and A. G. Sykes, *J. Am. Chem. Soc.*, **97**, 5918 (1975).
(2) M. Ardon and A. Pernick, *J. Am. Chem. Soc.*, **95**, 6871 (1973); **96**, 1643 (1974).
(3) (a) P. Souchay, M. Cadiot, and M. Duhameaux, *C. R. Acad. Sci.*, **262**, 1524 (1966); (b) P. Souchay, M. Cadiot, and B. Viossat, *Bull. Soc. Chim. Fr.*, **3**, 892 (1970).
(4) For a striking example illustrating the reliability of this method cf. (a) W. P. Griffith, W. P. Lewis, and G. Wilkinson, *J. Inorg. Nucl. Chem.*, **7**, 38 (1958); (b) M. Ardon, *Bull. Res. Counc. Isr., Sect. A*, **11**, 183 (1962); *Isr. J. Chem.*, **2**, 181 (1964); (c) R. D. Feltham, *Inorg. Chem.*, **3**, 1038 (1964); (d) B. F. Hoskins, F. B. Whillans, D. H. Dale, and D. C. Hodgkin, *Chem. Commun.*, 69 (1969).
(5) Ordinary cryoscopy (in water) as well as cryoscopy in a saturated solution of Na_2SO_4 (salzkryoskopie) are not suitable for this purpose (cf. ref 8).
(6) (a) M. Ardon and A. Linenberg, *J. Phys. Chem.*, **65**, 1443 (1961); **76**, 4040 (1972); (b) M. Ardon and L. Halicz, *Inorg. Chem.*, **12**, 1903 (1973).
(7) M. Ardon and A. Pernick, *Inorg. Chem.*, **12**, 2484 (1973).
(8) M. Ardon and G. Yahav, *Inorg. Chem.*, **15**, 12 (1976).

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Selenium Protection against Mercury Toxicity. Binding of Methylmercury by the Selenohydryl-Containing Ligand

Sir:

Since the hitherto unknown Minamata disease was identified as methylmercury intoxication from contaminated fish, mercury compounds, in particular methylmercury, have drawn considerable attention in scientific circles. In detoxification of heavy metals such as cadmium and mercury, the role of metallothionein, a sulfhydryl-rich protein, has been frequently implied.[1] Recent NMR investigations indicate that of the potential binding sites in cysteine, penicillamine, and glutathione, the sulfhydryl group binds methylmercury most strongly.[2] Ganther et al.[3] reported that selenium and mercury tend to accumulate together in tuna, and adequate protection against mercury toxicity has been avidly researched. At present, some of the most promising protective agents against acute mercury toxicity are the seleni-

Table I. Proton Chemical Shift and Mercury-Proton Coupling Constant for Methylmercury Complexes of Selenocysteamine and Cysteamine

Donor atom (X)	Complex formula	Chemical shift, ppm[a] (1) (2) $HX-CH_2-CH_2-NH_3^+$ $CH_2(1)$ $CH_2(2)$	$J_{^{199}Hg-^1H}$, cps	ΔCH_3Hg-,[b] cps
Se	$HSe(CH_2)_2NH_3^+$	2.72 3.17		
	$CH_3HgSe(CH_2)_2NH_3^+$	3.05	162.0	12.4
S	$HS(CH_2)_2NH_3^+$	2.80 3.16		
	$CH_3HgS(CH_2)_2NH_3^+$	3.14	185.7	8.2
	CH_3HgCl		218.8	0

[a] Chemical shift was measured from internal DSS. [b] The value represents the difference in chemical shift between methylmercury only and its complex.

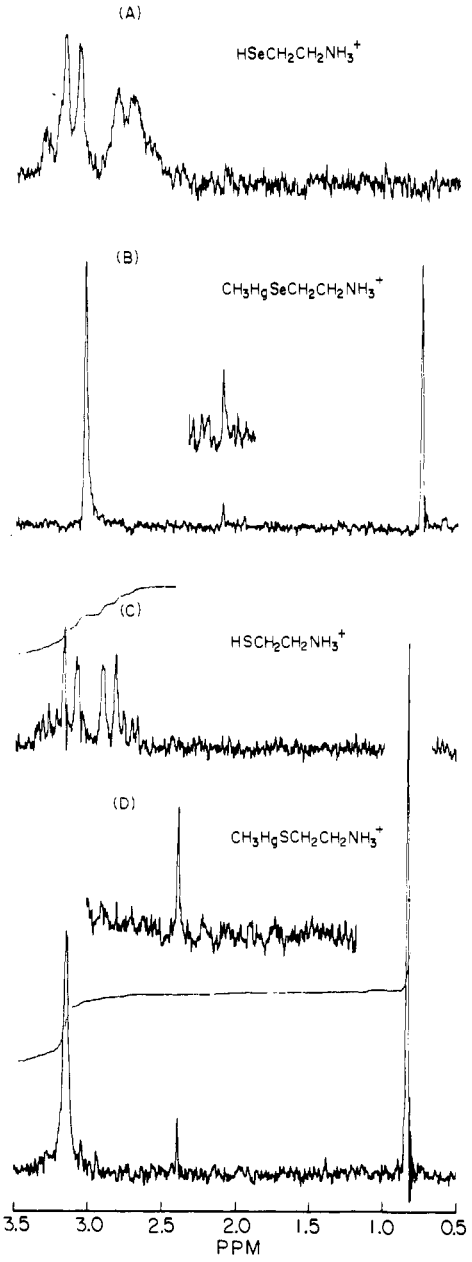

Figure 1. ^1H NMR spectra of selenocysteamine (A), selenocysteamine-methylmercury complex (B), cysteamine (C), and cysteamine-methylmercury complex (D).

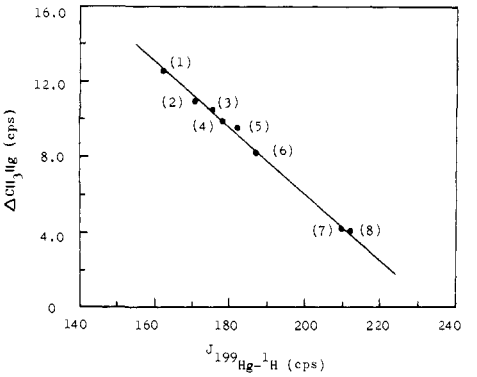

Figure 2. Plot of chemical shift of methyl group vs. $^{199}Hg-^1H$ coupling constant of methylmercury complexes. The numbers represent the methylmercury complexes of the following ligands: (1) selenocysteamine; (2) glutathione (see ref 2a); (3) penicillamine; (4) cysteine (see ref 2a); (5) 2-mercaptopropionylglycine; (6) cysteamine; (7) 1,4-butanediamine; (8) 1,3-propanediamine.

um compounds.[4] The importance of the binding of methylmercury by the selenium donor is well known, however, the interaction has never been quantitatively characterized. We investigated the binding of methylmercury by the selenohydryl group using ^1H NMR spectroscopy and comparisons with binding by sulfhydryl and amino groups.

Selenocysteamine was synthesized according to Klayman's method[5] and freshly used, as it readily oxidizes to diselenide. The selenocysteamine-methylmercury (1:1) complex was isolated, and the results of microanalysis and infrared measurement[6] indicated the molecular formula, $CH_3HgSeCH_2CH_2NH_3^+Cl^-$: Anal. Calcd for $C_3H_{10}NSeHgCl$: C, 9.61; H, 2.69; N, 3.73. Found: C, 10.02; H, 3.04; N, 3.91. Proton magnetic resonance spectra were measured in 50% dioxane-d_8 at 60 MHz on a Varian spectrometer, Model A-60.[7]

The binding of methylmercury by the ligands was characterized by monitoring the chemical shift of the methyl protons of methylmercury, the mercury-proton coupling constant ($J_{^{199}Hg-^1H}$)[8], and the chemical shifts of the ligand protons. Figure 1 shows typical ^1H NMR spectra of selenocysteamine, cysteamine, and their methylmercury complexes at pD 3.6.[9] The values of the coupling constants and chemical shifts are summarized in Table I. The most striking feature is the small $J_{^{199}Hg-^1H}$ value of the selenocysteamine-methylmercury complex, namely, the high affinity of the selenohydryl group to methylmercury in comparison with that of the sulfhydryl group. Figure 2 shows the linear correlation between the coupling constants and the chemical shifts of the methyl resonances in the methylmercury complexes coordinated by selenohydryl, sulfhydryl, and amino groups. The order ($Se > S > NH_2$) of affinity to methylmercury is the reverse of that expected from the basicity of the donor groups. The ionization constants estimated from potentiometric and ^1H NMR titrations were $pK(SeH) = 5.0$ and $pK(NH_3^+) = 10.8$ for selenocysteamine and $pK(SH) = 8.3$ and $pK(NH_3^+) = 10.5$ for cysteam-

ine at 20° and μ = 0.1. In general, the basicity of the SeH group is considerably lower than that of the SH group. On the other hand, there is a fair correlation between the mercury-proton coupling constants and the electronegativity (Se, 2.4; S, 2.5; N, 3.0) or covalent radius (Se, 1.16; S, 1.02, N, 0.75 Å) of the coordination atoms. These results suggest high covalency of the CH_3Hg–Se bond which involves d_π–d_π back-bonding. In view of the fact that selenium compounds afford excellent protection against mercury intoxication,[10] our finding herein that the selenohydryl group has a high affinity for the mercury compound is of particular interest.

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References and Notes

(1) T. W. Clarkson, *Annu. Rev. Pharmacol.*, **12**, 375 (1972); J. H. Kägi, S. R. Himmelhoch, P. D. Whanger, J. L. Bethune, and B. L. Vallee, *J. Biol. Chem.*, **249**, 3537 (1974).
(2) (a) D. L. Rabenstein and M. T. Fairburst, *J. Am. Chem. Soc.*, **97**, 2086 (1975); (b) Y. Hojo, Y. Sugiura, andH. Tanaka, *J. Inorg. Nucl. Chem.*, **38**, 641 (1976).
(3) H. E. Ganther, C. Goudie, M. L. Sunde, M. J. Kopecky, P. Wagner, S.-H. Oh, and W. J. Hoekstra, *Science*, **175**, 422 (1972).
(4) I. Bremner, *Q. Rev. Biophys.*, **7**, 75 (1974).
(5) D. L. Klayman, *J. Org. Chem.*, **30**, 2454 (1965).
(6) In the infrared spectrum of the isolated complex, the presence of $\nu(NH_3^+)$ near 3000 cm^{-1} and the disappearance of ν(SeH) at 2575 cm^{-1} in the free ligand revealed that selenocysteamine coordinates to methylmercury through the selenium atom only, consistent with a methylmercury coordination number of one.
(7) The sample was prepared by mixing the D_2O solution of the ligand (0.2 M) and the dioxane-d_8 solution of methylmercuric chloride (0.2 M) just before the ^1H NMR measurements, as the sample solution showed signs of precipitation after a few minutes.
(8) J. V. Hatton, W. G. Schneider, and W. Siebrand, *J. Chem. Phys.*, **39**, 1330 (1963). The satellite lines of methylmercury are due to methyl groups bonded to mercury-199 (I = ½, natural abundance 16.9%) while the central resonance is due to methyl groups bonded to all other isotopes of mercury.
(9) The pH independence of the chemical shifts and coupling constant in an equimolar solution of methylmercury and selenocysteamine (or cysteamine) indicates the complex formation of methylmercury by the ligand over the pH range 1–12.
(10) The action of selenocysteamine on mercury toxicity was investigated in male Wister rats (ca. 150 g) in a dose of 10 μmol/kg sc. The percentage of living animals was 20% in those treated with the mercury compound only (20 μmol/kg ip). A concurrent treatment of selenocysteamine resulted in an increase to 90%.

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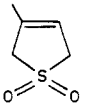
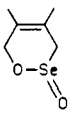
(2 + 4) (π + $\pi\pi$) and (2 + 4) (n + $\pi\pi$) Modes of Addition in the Reaction between SO_2 and a Diene. Kinetic vs. Thermodynamic Control

Sir:

Since de Bruin suggested a cyclic sulfone structure **1** for the crystalline monoadduct obtained from the reaction of liquid sulfur dioxide and isoprene at room temperature,[1] subsequent investigations, conducted in part by Backer and Strating in our laboratories,[2] proved this assumption to be correct and many examples of this 1,4-cycloaddition to dienes have been reported. The industrial value of this type of reaction can be judged from the fact that the sulfur dioxide addition to butadiene is the key step in the synthesis of sulfolane. Although selenium dioxide was at first expected to react with 1,3-dienes in an analogous way, it was show by ^1H NMR spectroscopy that cyclic seleninic esters[3] (e.g., **2**) are formed, thus indicating that (2 + 4) (π + $\pi\pi$) instead of (2 + 4) (n + $\pi\pi$) reactions[4] had taken place between the dienes and selenium dioxide. This difference in chemical behavior combined with the fact that rearrangements of sulfinic esters to sulfones[5] were likely to occur under the conditions employed for almost all sulfur dioxide additions,[6] prompted us to investigate the addition of both dioxides to 1,2,5,6-tetramethyl-3,4-dimethylenetricyclo[3.1.0.0^{2,6}]hexane **3**,[7] which had been shown before to be highly reactive in Diels–Alder processes.[8]



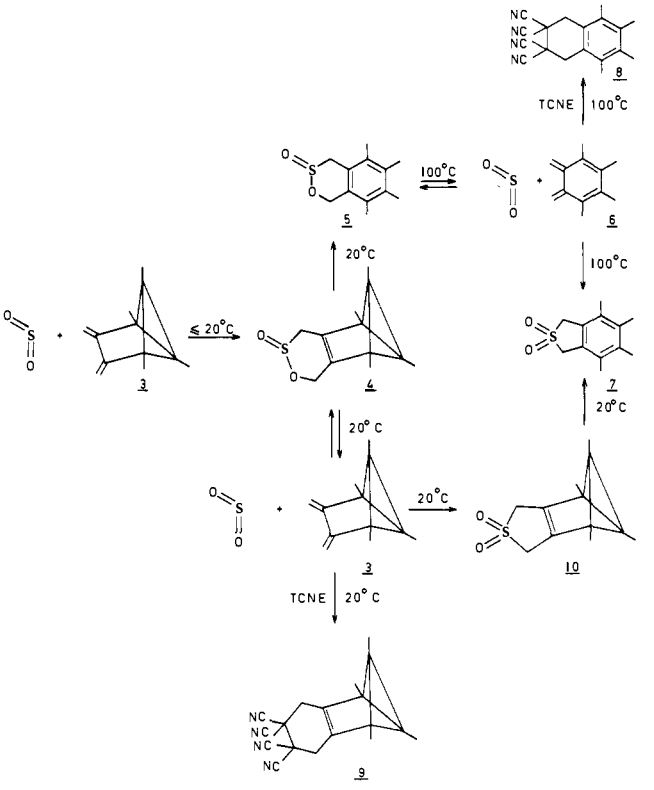
1



2

When sulfur dioxide (dried over phosphorus pentoxide) was introduced at or below room temperature into a stirred chloroform[9] solution of tricyclic diene **3**, an instantaneous reaction took place leading to the kinetically-controlled formation of the sulfinic ester **4**[10] by a (2 + 4) (π + $\pi\pi$) cycloaddition. The ester is not stable at room temperature and rearranges thermally via two possible pathways, namely, preferentially (isolated 90%) to the aromatic ester **5**[11] by an isomerization of the bicyclobutane moiety, and to sulfone **10**[12] (Scheme I).[13]

Scheme I



Heating the aromatic ester **5** in *o*-dichlorobenzene at 80–100 °C caused dissociation into sulfur dioxide and the *o*-xylene derivative **6**, followed by recombination[14] yielding in a thermodynamically controlled (2 + 4) (n + $\pi\pi$) cycloaddition the aromatic sulfone **7**[15] (98% isolated). This sulfone was also obtained by isomerization of the bicyclobutane moiety in sulfone **10** at room temperature.

The thermal rearrangement of cyclic β,γ-unsaturated sulfinic esters to sulfones has been shown to proceed via a retro-Diels–Alder reaction.[14] Confirmation of this cycloreversion has now been obtained in the case of both the polycy-